

# Online Research @ Cardiff

This is an Open Access document downloaded from ORCA, Cardiff University's institutional repository: <https://orca.cardiff.ac.uk/id/eprint/106307/>

This is the author's version of a work that was submitted to / accepted for publication.

Citation for final published version:

Balasundaram, S. V., Hess, J., Durling, M. B., Moody, S. C., Thorbek, L., Progidia, C., LaButti, K., Aerts, A., Barry, K., Grigoriev, I. V., Boddy, L. ORCID: <https://orcid.org/0000-0003-1845-6738>, Högberg, N., Kauserud, H., Eastwood, D. C. and Skrede, I. 2018. The fungus that came in from the cold: dry rot's pre-adapted ability to invade buildings. ISME Journal 12 , pp. 791-801. 10.1038/s41396-017-0006-8 file

Publishers page: <https://doi.org/10.1038/s41396-017-0006-8>  
<<https://doi.org/10.1038/s41396-017-0006-8>>

Please note:

Changes made as a result of publishing processes such as copy-editing, formatting and page numbers may not be reflected in this version. For the definitive version of this publication, please refer to the published source. You are advised to consult the publisher's version if you wish to cite this paper.

This version is being made available in accordance with publisher policies.

See

<http://orca.cf.ac.uk/policies.html> for usage policies. Copyright and moral rights for publications made available in ORCA are retained by the copyright holders.



The fungus that came in from the cold: Dry rot's pre-adapted ability to invade buildings

S. V. Balasundaram<sup>1</sup>, J. Hess<sup>1</sup>, M. B. Durling<sup>2</sup>, S. C. Moody<sup>3</sup>, L. Thorbek<sup>1</sup>, C. Progida<sup>1</sup>, K. LaButti<sup>4</sup>, A. Aerts<sup>4</sup>, K. Barry<sup>4</sup>, I. V. Grigoriev<sup>4</sup>, L. Boddy<sup>5</sup>, N. Högberg<sup>2</sup>, H. Kauserud<sup>1</sup>, D. C. Eastwood<sup>3</sup>, I. Skrede<sup>1\*</sup>

<sup>1</sup>Department of Biosciences, University of Oslo, Oslo, Norway; <sup>2</sup>Department of Forest Mycology, Swedish Agricultural University, Uppsala, Sweden; <sup>3</sup>Department of Biosciences, Swansea University, Swansea, UK; <sup>4</sup> United States Department of Energy Joint Genome Institute, Walnut Creek, CA, USA; <sup>5</sup>School of Biosciences, Cardiff University, Cardiff, UK;

Correspondence and request for materials should be addressed to I.S.  
([inger.skrede@ibv.uio.no](mailto:inger.skrede@ibv.uio.no))

21    Abstract

22    Many organisms benefit from being pre-adapted to niches shaped by human activity, and  
23    have successfully invaded man-made habitats. One such species is the dry-rot fungus *Serpula*  
24    *lacrymans*, which has a wide distribution in buildings in temperate and boreal regions, where  
25    it decomposes coniferous construction wood. Comparative genomic analyses and growth  
26    experiments using this species and its wild relatives revealed that *S. lacrymans* evolved a  
27    very effective brown rot decay compared to its wild relatives, enabling an extremely rapid  
28    decay in buildings under suitable conditions. Adaptations in intracellular transport  
29    machineries promoting hyphal growth, and nutrient and water transport may explain why it is  
30    has become a successful invader of timber in houses. Further, we demonstrate that *S.*  
31    *lacrymans* has poor combative ability in our experimental set-up, compared to other brown  
32    rot fungi. In sheltered indoor conditions, the dry rot fungus may have limited encounters with  
33    other wood decay fungi compared to its wild relatives. Overall, our analyses indicate that the  
34    dry rot fungus is an ecological specialist with poor combative ability against other fungi.

35

## 36 Introduction

37 Species worldwide are negatively affected by anthropogenic habitat destruction.  
38 However, for those few species originally living in natural habitats that resemble the man-  
39 made ecosphere, the opposite is also the case. Animals like the Norwegian rat (*Rattus*  
40 *norvegicus*) and the German cockroach (*Blatella germanica*) have extended their distribution  
41 dramatically (Robinson, 1965; Nentwig, 2008). Likewise, many plant pathogenic fungi have  
42 become extremely widespread as monotypic crop cultivation creates large habitats, and the  
43 trade and transport of these crops aid their dispersal ( Anderson *et al.*, 2004; Grunwald *et al.*,  
44 2008; Stukenbrock *et al.*, 2011). A similar pattern is seen with the few wood decay fungi that  
45 have expanded their realized niche into the human built environment.

46 Probably the best-known example of a successful fungal invader of the built  
47 environment is the dry rot fungus *Serpula lacrymans* var. *lacrymans* (subsequently referred  
48 to as var. *lacrymans*), which is distributed in houses in temperate and boreal regions  
49 worldwide causing brown rot decay. It spreads with human transport of timber over long  
50 distances and colonizes new buildings in its vicinity by air-borne spores (Kausrud *et al.*,  
51 2007; 2012). Colonization of construction timber in buildings is characterised by rapid  
52 vegetative mycelial growth and formation of thick (up to 2 cm diameter, Figure 1) mycelial  
53 cords that mediate the transport of nutrition and water to new wood substrates (Jennings and  
54 Bravery, 1991). This allows quick growth and reallocation of resources via the transport of  
55 nutrition and water to the new wood substrates (Jennings and Bravery, 1991; Boddy *et al.*,  
56 2007).

57 Comparative genomic approaches have shown that var. *lacrymans* and other brown  
58 rot fungi have a reduced set of plant cell wall hydrolysing enzymes to decompose wood  
59 compared to the ancestral white-rot fungi ( Eastwood *et al.*, 2011; Arantes and Goodell,

2014; Riley *et al.*, 2014; Floudas *et al.*, 2015; Zhang *et al.*, 2016). A recent study has suggested that the set of secreted enzymes responsible for decomposition of var. *lacrymans* is even smaller than that of some other brown rot fungi (Presley and Schilling, 2017). The loss of enzymes by brown rot fungi is correlated with a strategy in which the initial attack of the wood is mediated by hydroxyl radicals produced by chelator-mediated Fenton (CMF) chemistry (Eastwood *et al.*, 2011; Floudas *et al.*, 2012; Riley *et al.*, 2014). These initial attacks have been suggested to be controlled by differential gene expression of the fungi (Zhang *et al.*, 2016; Presley and Schilling, 2017). The attacked wood structure is then further depolymerised by oxidising and hydrolysing enzymes that target cellulose and hemicellulose elements in the wood, while leaving modified lignin behind.

Var. *lacrymans* has a scattered natural range in high altitude mountain regions of North-East Asia, thriving in moraine-dominated habitats around the treeline where woody resources are heterogeneously distributed (Kausrud *et al.*, 2012). Human transport of infected wood appears to have facilitated the colonization in the human domain in temperate regions world-wide. It is widespread in buildings in Europe and Japan, and it is also found in buildings in temperate parts of North and South America (Chile), Australia and New Zealand, but with less abundance (White *et al.*, 2001; Kausrud *et al.*, 2007). The large European house-colonizing population of var. *lacrymans* has low genetic variation (Kausrud *et al.*, 2007; Skrede *et al.*, 2013), suggesting a severe population bottleneck during the colonisation of the European built environment (Kausrud *et al.*, 2012; 2007). *Serpula lacrymans* var. *shastensis* (subsequently referred to as var. *shastensis*) is a close relative of var. *lacrymans*, from high altitude mountain regions in the Cascade mountain range (North America), but has not been reported in the built environment (Harmsen, 1960; Palfreyman *et al.*, 2003). Although genetically well-separated, the two sub-species are able to form a dikaryotic mycelium when paired *in vitro* (Palfreyman *et al.*, 2003; Kausrud *et*

85 *al.*, 2007; Skrede *et al.*, 2011). In the habitat close to the treeline in the Cascades (Figure 1),  
86 var. *shastensis* colonizes and decays large logs of *Abies magnifica* (Kausrud *et al.*, 2004;  
87 2012). Both varieties of *S. lacrymans* appear to be ecological specialists, thriving in exposed  
88 mountainous habitats with patchy resource distribution.

89 In contrast to the confined niches of *S. lacrymans*, its sister species *Serpula*  
90 *himantoides* has a widespread circumboreal distribution in natural habitats in temperate and  
91 boreal regions (Carlsen *et al.*, 2011). As with *S. lacrymans*, *S. himantoides* causes brown rot  
92 of conifers, but decomposes wood more slowly, as shown on spruce (Harmsen, 1960), and  
93 produces smaller fruit bodies and smaller cords. *Serpula himantoides* is rarely found in  
94 buildings, and when it is, it decomposes wood more slowly than var. *lacrymans*. Unlike var.  
95 *lacrymans*, indoor colonization by *S. himantoides*, as with the majority of other wood-decay  
96 fungi, represent random, and repeated colonisations from nature (Kausrud *et al.*, 2012).

97 It is not evident which characteristics have made var. *lacrymans* such a successful  
98 invader of the built environment compared to its wild relatives. Pinpointing contrasting  
99 genomic differences among the lineages is a first step towards detecting the genetic basis of  
100 var. *lacrymans* invasiveness and persistence. In this study we, therefore, set out to reveal  
101 which genomic features separate var. *lacrymans* from its predominantly wild relatives. We  
102 analysed which genes have undergone shifts in selective pressure and then, which gene  
103 families have expanded or contracted during divergence between variants or species. This  
104 was achieved by sequencing and *de novo* genome assembly of var. *lacrymans* and var.  
105 *shastensis* strains and comparing these to the genome of the sister species *S. himantoides*.  
106 Genomic analyses were complemented by two growth experiments investigating differences  
107 in decomposition ability and interspecific competition, to provide more direct evidence for  
108 how each of these factors may contribute to var. *lacrymans*' success in the built environment.

## Materials and Methods

*Strains.* Three strains were used for physiological experiments and genome comparisons in this study. The *S. himantoides* strain (MUCL38935) was cultured from soil in the UK in 1994, the var. *shastensis* strain (SHA17-1) was collected in California, US on *Abies* in 2004 and the var. *lacrymans* (SL200) was collected from a house in Poland in 1953. Since these strains have been maintained in culture for extended periods of time, caution should be used when interpreting the results as the strains may have changed their behaviour through these years.

*DNA extraction, sequencing assembly and gene predictions.* More details of the DNA extraction, library preparation, sequencing procedure, and gene prediction pipeline can be found in the Supplementary text. DNA of all three strains was extracted by a modified phenol-chloroform protocol available at the JGI webpage (<http://jgi.doe.gov/collaborate-with-jgi/pmo-overview/protocols-sample-preparation-information/>). All strains were sequenced using Illumina technology. The two *Serpula lacrymans* strains were sequenced on a Illumina GAII at the SNP&SEQ Technology Platform in Uppsala, Sweden, while *S. himantoides* was sequenced on a Illumina Hiseq 2000 at the JGI ([http://genome.jgi.doe.gov/Serla\\_varsha1/Serla\\_varsha1.info.html](http://genome.jgi.doe.gov/Serla_varsha1/Serla_varsha1.info.html)).

The Velvet *de novo* assembler (Zerbino and Birney, 2008) was used to assemble reads into contigs for var. *lacrymans* and var. *shastensis*. JGI assembled *S. himantoides* with the AllPathsLG assembler (Gnerre *et al.* 2011). The CEGMA pipeline was used (Parra *et al.*,

2007) to estimate completeness of all assemblies (Table 1). Protein coding genes in the three *Serpula* strains were annotated using MAKER2 version 2.27 (Holt and Yandell, 2011).

*Functional annotation.* Genes were given a preliminary description by BLAST alignment towards Uniprot. InterProScan was used for functional annotation and classifications of protein families (Jones *et al.*, 2014). Protein sequences of var. *lacrymans*, var. *shastensis* and *S. himantioides* were obtained from the MAKER2 predictions.

*OrthoMCL clustering.* Homologous proteins of the three *Serpula* strains were clustered using the software OrthoMCL (Li *et al.*, 2003). This tool clusters homologous proteins across the given species using Markov cluster algorithm to group orthologs and paralogs. In total 34,273 protein sequences from three different *Serpula* genomes were compared.

*CAFÉ analysis.* CAFÉ estimates a global birth and death rate of gene families and changes in gene family size across a phylogeny (De Bie *et al.*, 2006). All orthoMCL clusters were used as gene families. CAFÉ was run using a global birth/death parameter ( $\lambda$ ). Rapidly evolving gene families were estimated using the best fit  $\lambda$  (0.002) at a p-value threshold of 0.01. The ultrametric tree used for CAFÉ analysis was based on a multi-locus maximum likelihood phylogeny of ten loci from (Balasundaram *et al.*, 2015) that was made ultrametric in the R package APE (Paradis *et al.*, 2004).



*Selection pressure.* Clusters of single copy orthologs were chosen to screen for branch specific changes in selection pressure. The clusters were aligned with the multiple sequence alignment program PRANK (Loytynoja, 2014) with the ‘codon’ alignment mode, using the species phylogeny (Skrede *et al.*, 2011) as guide tree. PRANK has been shown to provide the most accurate alignments, with the lowest false-positive rates (Fletcher and Yang, 2010). The Codeml from the PAML package (Yang, 2007) was used to identify changes in selection regime. For each group of orthologs, a single dN/dS ratio ( $\omega$ ) was estimated for all branches on the tree ( $H_0$ ) and for three instances where each one of the species was allowed to evolve at a separate rate ( $H_1$ ). The best fit model was determined using a likelihood ratio test and p-values were adjusted to control the false discovery rate (FDR) for multiple hypothesis testing using a  $\alpha < 0.05$  (Benjamini *et al.*, 2006). All alignments with a significant shift in selection pressure between species were manually examined to remove questionable alignment regions if present and were then rerun in the above outlined analysis.

*Functional enrichment analyses.* Functional enrichment analysis was used to characterize the genes present in all the genomes compared to gene families that were inferred to be expanded or contracted by CAFÉ. A Python script was used to perform functional enrichment analysis of PFAM domains using Fisher’s exact test (<http://cgrlucb.wikispaces.com/Functional+Enrichment+Analysis>).

*Annotation of genes of specific functions of interest.* To predict the secretome of each species, a bioinformatics pipeline consisting of SignalP 4.1 (Petersen *et al.*, 2011), TargetP 2.0 (Emanuelsson *et al.*, 2007), TMHMM 2.0 (Krogh *et al.*, 2001), PS\_scan (Hulo, 2006) and WolfPSort v. 0.2 (Horton *et al.*, 2007), was used, as implemented in Kohler *et al.* (Kohler *et*

178 *al.*, 2015). Besides the annotations generated for the entire proteomes (e.g. CAZymes, PFAM  
179 domains), the proteolytic enzymes present in each secretome were also annotated through  
180 BLAST searches against the MEROPS database (Rawlings *et al.*, 2014). Carbohydrate-active  
181 enzymes were predicted by searching predicted proteomes with the dbCAN tool (Yin *et al.*,  
182 2012; Lombard *et al.*, 2014).

183         As cytochrome P450 (cytP450) is an important class of enzymes involved in  
184 specialized metabolism, the clusters annotated with cytP450 PFAM domains in Interproscan  
185 were manually curated. Only those of over 300 residues with both the EXXR and CXG motif  
186 were accepted as functional, according to the method of Syed and Mashele (2014). According  
187 to cytP450 nomenclature, a similarity of 40% was considered sufficient to classify a  
188 predicted protein into a particular family. A similarity of 55% would allow allocation to a  
189 sub-family. Those with <40% similarity to named cytP450s were – with those that had no  
190 significant matches in the NCBI or Uniprot databases – considered to probably belong to  
191 novel cytP450 families.

192  
193         *Data availability.* All raw sequence reads, and assembled genomes are available on  
194 NCBI at Bioproject PRJNA412961. In addition, the *S. himantoides* MUCL38935 genome is  
195 available at the JGI genome browser  
196 ([http://genome.jgi.doe.gov/Serla\\_varsha1/Serla\\_varsha1](http://genome.jgi.doe.gov/Serla_varsha1/Serla_varsha1)). The MAKER2 gene predictions,  
197 the OrthoMCL clusters and the alignments used as input to the Codeml analyses have been  
198 deposited in the Dryad Digital Repository: <http://dx.doi.org/xxxx>.

200            *Combative ability*. Var. *lacrymans*, which is found predominately, if not exclusively,  
201 inside houses in Europe, was hypothesized to show decreased ability to combat for limited  
202 resources since it faces few competitors in this environment. An antagonistic experiment was  
203 used to test this hypothesis, where the three *Serpula* strains of interest were confronted with  
204 each other and other brown rot decomposer fungi, pairwise, by growing two well-colonized  
205 blocks side by side (see supplementary text for detailed experimental setup). The three  
206 *Serpula* strains, and the three species *Antrodia xantha*, *Coniophora puteana* and *Fomitopsis*  
207 *pinicola* were used. All combinations were repeated 10 times. After the experiment, three  
208 small wood pieces from within the wood block were transferred to three new culture plates.  
209 The strains that were re-isolated from the wood piece were identified and reported. A  
210 Pearson's  $\chi^2$  Goodness of Fit test was used to test whether one species had significantly  
211 outcompeted another.

212  
213            *Wood decay*. The specialized house-living var. *lacrymans* was expected to decompose  
214 spruce especially fast as it is mostly found on spruce in houses, where it is known to grow  
215 quickly (Harmsen, 1960). To compare the decomposition ability of the three *Serpula* strains  
216 and *A. xantha*, *F. pinicola* and *C. puteana*, mass loss of wood was determined after 60 days  
217 colonization at 20 °C on the three tree species *Pinus sylvestris*, *Picea abies* and *Abies*  
218 *lasiocarpa* (See supplementary text for experimental setup; the three non-*Serpula* species  
219 were not tested on *Abies lasiocarpa*). The significance of the differences in mass loss among  
220 strains and among wood species was tested with ANOVA analyses using R (R Core Team,  
221 2008).

## Results

*Genome summary.* The gene prediction pipeline identified a total of 11,352 gene models in var. *lacrymans*, 10,910 gene models in var. *shastensis* and 12,011 gene models in *S. himantioides* (Table 1). Annotated genes were clustered into gene families, of which 6,695 were shared among all three strains, corresponding to approximately 55% to 61% of annotated genes in each genome. Given the close relationship among the three species, the number of singleton clusters inferred for each species was surprisingly high. Of the predicted genes 18% in var. *shastensis*, 23% in var. *lacrymans* and 24% in *S. himantioides* were unique to each of the three lineages. Further analysis of singleton genes showed that singletons predominately represented cases where orthologs were absent in the other two species, either due to gene loss or absence of the corresponding coding region from the respective assemblies (results not shown).

*Analyses of selection.* The genome-wide estimates of selection yielded a mean estimate of  $\omega = 0.137$  for *S. himantioides*,  $\omega = 0.179$  for var. *lacrymans* and  $\omega = 0.234$  for var. *shastensis* (gene clusters with  $\omega > 2$  were omitted from these estimates).

Shifts in selective pressure on individual genes between species may pinpoint genes whose functions have contributed to adaptation by each species to their respective realised niches. For the analyses of shifts in selective pressure on a gene-by-gene basis, three series of tests were run, each one with a different species as the foreground branch. After correction for multiple testing, 100, 129, and 265 genes with significantly different  $\omega$  between foreground and background branches in var. *lacrymans*, var. *shastensis* and *S. himantioides* were detected, respectively (Figure 2). Among the sets of genes, 43% were annotated with

PFAM domains while the rest were unannotated. Our functional analyses were only focused on the genes that had PFAM annotations. A full list of significant genes is provided in the supplementary material (Supplementary Table 1).

One of the most pronounced functional signatures detected was the selective shift in many proteins involved in intracellular transport (Table 2) with an elevated  $\omega$  in *S. lacrymans* compared to *S. himantioides* (higher  $\omega$  in one or both of the *S. lacrymans* varieties). Several of these proteins identified were involved in the transport of vesicles to the Golgi stack for secretion, (see supplementary text for details). In contrast, a protein involved in early endosomal membranes evolved faster in *S. himantioides* than in *S. lacrymans*. This, suggested a faster evolution of an endocytic pathway in *S. himantioides* versus an exocytic pathway in *S. lacrymans*.

In addition to the genes related to membrane transport, two regulators of actin polymerization (the guanine nucleotide exchange factors, Rho GEF and Ras GEF) and a gene with a role in actin depolymerization (cofilin) evolved significantly faster in var. *lacrymans* than in *S. himantioides* (Table 2).

*Expansion and contraction of gene families.* All clusters in the dataset and a rooted tree were used to infer 244 and 262 gene families that were expanded on the var. *lacrymans* branch and on the var. *shastensis* branch, respectively, compared to the rest of the tree (Table 2). Only 5 were expanded on the common branch leading to var. *lacrymans* and var. *shastensis*. Compared to the genomic background, CAFÉ inferred 112 and 135 gene families that expanded significantly faster than expected (based on all clusters) in var. *lacrymans* and var. *shastensis*, respectively (P-value 0.01). In turn, 596 and 473 gene families were contracted on the var. *lacrymans* branch and on the var. *shastensis* branch, respectively, and

332 were contracted on the common branch. Six (var. *lacrymans*) and four (var. *shastensis*) gene families showed significantly higher rates of contraction than the genomic background rate.

Functional enrichment of the expanded and contracted gene families demonstrated a change in copy number for gene families related to specialised metabolism amongst all three strains (Supplementary Table 2). In particular, expansions and contractions in a variety of polyketide synthase (PKS) and nonribosomal peptide synthase (NRPS) related PFAM domains were identified (Supplementary Table 2). One NRPS gene family (cluster 0012) was expanded in var. *lacrymans*, var. *shastensis* and their common branch. This gene family had nine gene copies in var. *shastensis* and var. *lacrymans*, but only one in *S. himantioides*. The opposite pattern was found for a putative PKS-NRPS hybrid protein gene family of unknown function (cluster 0005), where *S. himantioides* had ten copies, var. *lacrymans* eight and var. *shastensis* six copies. Copy number changes in ATP-binding cassette (ABC) transporters were also detected. These were reduced in var. *lacrymans* compared to var. *shastensis* and *S. himantioides*.

Cytochrome P450s showed expansion in *S. lacrymans* compared to *S. himantioides* (Supplementary Table 3). Eighty-nine, 91 and 109 predicted functional cytochrome P450s were identified in *S. himantioides*, var. *shastensis* and var. *lacrymans* respectively. Thus, both var. *shastensis* and var. *lacrymans* have experienced expansion of capacity compared to *S. himantioides*, with an extra five families represented in each. Var. *lacrymans* and var. *shastensis* had the same families except that var. *shastensis* uniquely had one member of CYP5145, and var. *lacrymans* had one member of CYP6001, a family that was not present in either of the other strains. Thus, in both var. *shastensis* and var. *lacrymans* the higher numbers of cytochrome P450 copies were predominantly the result of an increased number of genes from existing families.

Several gene families related to wood decay mechanisms were expanded or contracted (Figure 2; Supplementary Table 2, supplementary text for details). Specifically, the set of CAZymes encoded within the three genomes was very similar, but with a somewhat greater gene complement in *S. himantioides* (see supplementary text for details; Supplementary Table 4). In contrast, an iron reductase with only a CBM1 and a CytB domain was found in *S. lacrymans*, but not in *S. himantioides*. (Supplementary Figure 2).

*Evaluating substrate preference.* Both *S. lacrymans* varieties decomposed more of the spruce wood block than *S. himantioides*, under the conditions tested (50% and 45% vs. 30% mass loss, respectively; Figure 3). There was no significant difference in the amount of decomposition between var. *lacrymans* and var. *shastensis* on spruce or fir ( $\chi^2$ ,  $p>0.05$ ). Var. *shastensis* failed to grow on pine, but it is unknown whether this is due to its inability to decompose pine, or due to other experimental factors, e.g. the experimental set-up on moist perlite may not have provided enough minerals. Spruce was more readily degraded by all strains, and this was particularly pronounced for var. *lacrymans*, which caused a mass loss of 50% of spruce but only 5% of pine wood blocks. See supplementary material for the mass loss of the additional species (Supplementary Figure 1).

*Evaluating antagonistic behavior.* *Serpula himantioides* was significantly more combative than var. *lacrymans* and var. *shastensis*, as well as the three other brown rot species under the conditions tested (Table 3). *Serpula himantioides* was present in 79% of the re-isolations from the confrontations against the other species (i.e. as 50% would be a deadlock, *S. himantioides* took the substrate of the other species in 29% of the cases). The two *S. lacrymans* varieties were less able to exclude the other species compared to *S.*

318 *himantioides* in this experiment, (var. *shastensis* was found in 40% and var. *lacrymans* in 41%  
319 of the cultures following confrontations, i.e. both lost their substrate in about 10% of the  
320 cases). When var. *lacrymans* and var. *shastensis* were confronted with *C. puteana* and *A.*  
321 *xantha*, the outcomes were close to 50% (i.e. a deadlock), but both *S. lacrymans* varieties  
322 were excluded by *S. himantioides* and *F. pinicola* (Table 3).



## 323 Discussion

324         In this study we aimed to identify which features have made the dry rot fungus  
325 *Serpula lacrymans* var. *lacrymans* the most successful invasive wood-decay fungus in the  
326 built environment by comparing its characteristics to its less invasive relatives. Since the  
327 successful establishment of an invasive species typically depends on a range of factors, we  
328 investigated the contribution of physiological factors (decomposition and combative ability),  
329 as well as underpinning genomic features. We detected numerous genomic signatures that  
330 may be linked to var. *lacrymans* invasiveness, including changes in selection pressure and  
331 evolution in gene families involved in hyphal growth, transportation, defence and  
332 decomposition of wood. Our experimental data suggest that *S. lacrymans* has poor  
333 antagonistic abilities towards other brown rot fungi, but that it has high wood-decomposition  
334 ability compared to its largely non-invasive relative *S. himantioides*. This suggests that *S.*  
335 *lacrymans* is an ecological specialist while *S. himantioides* is more of an ecological generalist.

336         One of our main findings is the differences in genome-wide selection pressure,  
337 evaluated by changes in rates of non-synonymous to synonymous substitutions ( $\omega$ ). The  $\omega$   
338 values suggested that on average the genes of *S. himantioides* experienced stronger purifying  
339 selection than those of var. *lacrymans*, and especially those of var. *shastensis*. However, even  
340 if  $\omega$  values can detect genes under selection, systematically increased  $\omega$  at the genome-wide  
341 level, can also be the result of demographic history (Tajima, 1989). In organisms with small  
342 effective population sizes, selection is less effective in removing deleterious mutations which  
343 can lead to elevated genome-wide  $\omega$  values. Correspondingly, we suggest that var.  
344 *lacrymans* and var. *shastensis*, which have higher average  $\omega$  overall in the genome, may  
345 have lower effective population sizes compared to *S. himantioides*. The differences in  
346 effective population size is expected as *S. himantioides* is distributed worldwide (Carlsen *et*

347 *al.*, 2011), while var. *shastensis* has limited current distribution and var. *lacrymans* has gone  
348 through a domestication process (Kausrud *et al.*, 2007).

349 In more detail, the genomic analyses revealed a selective shift in genes with functions  
350 involved in intracellular transport, growth and reorganization of the cell. Our data suggest  
351 that evolutionary changes to these processes may underlie the increase capacity of  
352 transportation and growth in var. *lacrymans* which in turn is likely to be a key factor for its  
353 success in the built environment. Buildings are a dry habitat, where the water resources are  
354 the most limiting factor. Var. *lacrymans* can produce the thickest mycelial cords described in  
355 the fungal kingdom, up to 2 cm in diameter (Jennings and Bravery, 1991). In comparison, var.  
356 *shastensis* and *S. himantoides* produce smaller cords, and *S. himantoides* has a slower  
357 growth rate (Harmsen, 1960). The corded network permits the translocation of intracellular  
358 resources, e.g. amino acids and water through vacuolar and vesicle trafficking to ensure  
359 complete exploitation of large woody substrates (Watkinson *et al.*, 2006).

360 Proteins associated with endomembrane system functioning and hyphal growth had  
361 different selection pressure between *S. lacrymans* and *S. himantoides*, indicating that  
362 changes in resource translocation are important in the adaptation to the different niches.  
363 Hyphal growth is dependent on both transport and fusion of secretory vesicles to the plasma  
364 membrane and on actin cytoskeleton organization and polarization. Indeed, actin is important  
365 for polarized growth and also represents the mechanism for the transport of secretory vesicles  
366 that contain materials for the synthesis of new cell wall and membranes in the growing tip  
367 (Berepiki *et al.*, 2011). We hypothesise that these genes play a role in the development and  
368 maintenance of the mycelial cords, possibly through mediating the re-grouping and re-  
369 allocation of resources.

370 To become a successful colonizer of wood, a fungus has to compete for resources  
371 with other decay species. However, the confrontation experiments, where the fungi were  
372 growing in fir blocks on moist perlite, revealed that var. *lacrymans* and var. *shastensis* have  
373 poor combative abilities compared to other wood decay fungi, at least in this nutrient poor  
374 set-up (Table 3). Species inhabiting more extreme environmental habitats may reduce their  
375 antagonistic abilities, following the universal adaptive strategy theory (Grime and Pierce,  
376 2012). Thus *S. lacrymans* inhabiting the dry treeline and built environments may have lost  
377 the capacity for broad antagonistic responses. This may also explain why var. *lacrymans*  
378 usually does not spread from colonized buildings into the natural environment, though a few  
379 exceptions have been noted in the Czech Republic (Kotlaba, 1992). In less stressful climates  
380 in the boreal and temperate zones, where *S. himantoides* is typically found, interspecific  
381 antagonistic interactions may be more important. Hence, under these conditions, it may have  
382 been more advantageous to evolve strong combative ability. This is supported by the  
383 increased numbers of PFAM domains possibly related to defence in *S. himantoides*  
384 compared to *S. lacrymans*, e.g. PKS and ABC transporters. PKS are large synthases  
385 particularly involved in the biosynthesis of specialized metabolites with many diverse  
386 functions. The gene families are known to expand and contract rapidly in response to  
387 adaptation to nutritional and environmental factors, pathogens or interactions with other  
388 organisms (Bushley and Turgeon, 2010). ABC transporters are often involved in the efflux of  
389 small metabolites ( Klein *et al.*, 2011; Karlsson *et al.*, 2015). Furthermore, similar expansions  
390 of PKS and ABC transporters have been observed in the mycoparasites *Clonostachys rosea*  
391 and *Trichodema virens*, and were suggested to be the reason for their extreme combative  
392 ability, by producing and transporting toxic compounds from the cells (Karlsson *et al.*, 2015).  
393 *Serpula himantoides* is known to produce antifungal substances, himanimides, that could  
394 increase its antagonistic ability (Aqueveque *et al.*, 2002). It is unknown if var. *lacrymans* can

produce these substances. More genomic analyses and experiments using different conditions are needed to pinpoint the exact function of the larger number of PKS and ABC transporters in *S. himantioides*, and whether any of these expansions are related to the previously detected himanimides.

Our growth experiments on wood substrates confirm earlier findings that var. *lacrymans* is a highly effective decomposer of coniferous wood (Harmsen, 1960). In natural environments, *S. lacrymans* typically occupies large logs of *Abies* or *Tsuga* (Figure 1) and has developed a unique capacity for rapid decay during a short season of favourable growth conditions. Resource availability and utilization of nutrients involve a diverse chemistry for saprotrophic fungi. The varying levels of extractives, such as terpenoids and other phenolic compounds, and the recalcitrant nature of the carbohydrates of wood imply that specialization and adaptation to these conditions are essential to utilize this niche. Our findings suggest that *S. lacrymans* is a more successful decomposer of spruce and fir than pine, and is more specialized for these specific substrates than *S. himantioides*. A more narrow substrate range was also suggested in a recent study of var. *lacrymans* and *Gloeophyllum trabeum*, where they found gene expression of a wider CAZyme complement in *G. trabeum* than in *S. lacrymans* (Presley and Schilling, 2017). Furthermore, the speed and efficiency with which *S. lacrymans* decomposes spruce, compared to *S. himantioides*, could be related to a more efficient CMF chemistry. The iron reductase (with a CBM1 domain and a cytochrome B domain) found in var. *lacrymans* and var. *shastensis*, but not *S. himantioides* has previously been suggested to have an electron transfer function (Yoshida *et al.*, 2005). Thus, it can target reduced iron directly to the cellulose substrate for efficient CMF. In previous analyses of *S. lacrymans*, this iron reductase was specifically pinpointed as important in the early oxidative degradation steps of the CMF chemistry (Eastwood *et al.*, 2011). This could contribute to more efficient utilization of carbohydrates from its habitat.

420 The content of inhibitory extractives is greater in pine wood than in spruce wood  
421 (Sjöström, 1993), which makes pine a less favourable food source for fungi. Differential gene  
422 expression analyses of a white rot fungus (*Phlebiopsis gigantea*) grown on wood where  
423 extractives were removed showed several genes potentially related to the processing of  
424 extractives (Hori *et al.*, 2014). These differentially expressed genes encoded glutathione-S  
425 transferase, ABC transporters, lipases, cytochrome P450s and aldehyde dehydrogenase. We  
426 found accelerated evolution in *S. lacrymans* for aldehyde dehydrogenase, an ABC transporter,  
427 and cytochrome P450s, and loss of copies of glutathione-S transferase and ABC transporters.  
428 The ability to process a diversity of extractives found in wood and secrete their breakdown  
429 products may, therefore, also play an important role in substrate specialization and hence  
430 adaptation of *S. lacrymans* to a different habitat. Furthermore, the loss of laccases and the  
431 increase of cytochrome P450s in the branch leading to *S. lacrymans* could be related to both  
432 community interactions and the processing of toxic phenolic derivatives produced during the  
433 decomposition of lignocellulose. Brown rot fungi do not utilize lignin, however, they  
434 depolymerize lignin to gain access to the cellulose and hemicellulose. Thus, as part of  
435 adapting to a specialized niche *S. lacrymans* may have lost genes important for exploitation  
436 of some woody substrates in nature, but rather specialized for a more streamlined  
437 decomposition of specific substrates. Cytochrome P450s have been suggested to easily  
438 duplicate, and to be important in the colonization of new environments and in the breakdown  
439 of novel compounds (Syed *et al.*, 2014). Moreover, it has been suggested that the large gene  
440 repertoire of cytochrome P450s evolved in *Phanerochaete chrysosporium* increased its  
441 resource availability (Syed and Yadav, 2012), thus the expansion of cytochrome P450s could  
442 be related to an expansion of biochemical capacity in var. *lacrymans* as it invades timber  
443 wood. Timber wood is similar to the wood encountered naturally by primary decay species,

444 containing more plant-derived compounds than partially degraded wood that is often  
445 available in the forest.

446         The chemistry of defence and foraging is a recurring issue in our dataset. However,  
447 without in-depth functional analysis, it is unclear whether the product moved by a particular  
448 ABC transporter or metabolised by a cytochrome P450 gene is of importance to the species'  
449 competitive ability and the decomposition of different substrates. Thus, further analyses of  
450 the increased set of cytochrome P450s in *S. lacrymans*, and the increased set of PKS and  
451 ABC transporters in *S. himantioides*, can pinpoint in which functions these gene expansions  
452 are involved.

453         Our results indicate that the devastating dry rot fungus is an ecological specialist that  
454 has developed highly effective brown rot decay and effective systems for transportation and  
455 growth. Common traits identified between genetically related var. *lacrymans* and var.  
456 *shastensis* when compared with the sister taxon *S. himantioides* suggest that var. *lacrymans*  
457 was pre-adapted to the built environment and that the requirements of the mountainous, dry,  
458 treeline habitat and the patchy nutrient environment of a house, including a blend of wood  
459 and mineral materials, share similar features important for *S. lacrymans*. This enabled var.  
460 *lacrymans* to opportunistically exploit the built environment when given the opportunity by  
461 human activity. Particularly, the evolution of the thick cords and rapid growth may be linked  
462 to its natural substrates, to maximize resource translocation and effectively decay the  
463 enormous logs. The lower combative ability, suggested from both physiological and genomic  
464 data and the narrower enzymatic assortment of our selected strains might explain why var.  
465 *lacrymans* rarely has been able to move from its new building niche back into temperate and  
466 boreal woodlands. As var. *shastensis* is very similar to var. *lacrymans* both in genetic and  
467 physiologic features, we conclude it has the potential to invade buildings, but has not done so

because its native range has not been widely exploited by humans and so has not been transferred to the built environment.

## Acknowledgements

We thank Ella Thoen for technical help, Anikó Várnai for discussions, and Skui Christmas Tree Plantation for the *Abies lasiocarpa* wood. BVS, JH, HK, LT and IS acknowledge the University of Oslo and Norwegian Research Council (project 221840) for funding. NS thanks the Swedish University of Agricultural Sciences and FORMAS (project 2010-1354) for funding. DE, SM and LB thank the UK Natural Environment Research Council, (award NE/K011588/1) for support. Sequencing of SL200 and SHA17-1 was performed by the SNP&SEQ Technology Platform in Uppsala. The facility is part of the National Genomics Infrastructure (NGI) Sweden and Science for Life Laboratory. The SNP&SEQ Platform is also supported by the Swedish Research Council and the Knut and Alice Wallenberg Foundation. The work conducted by the U.S. Department of Energy Joint Genome Institute, a DOE Office of Science User Facility, was supported by the Office of Science of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231.

## Conflict of interest

The authors declare no conflict of interest.

## Author contributions

I.S. J.H., H.K., D.C.E, N.H., and L.B. conceived and designed the research. L.T, I.S. and J.H. analysed physiological properties. I.S. extracted DNA. K.L., A.A. K.B. and I.V.G. sequenced

491 and analysed the *S. himantioides* genome at JGI. S.V.B., M.B.D., J.H., C.P. and S.C.M.  
492 analysed genomic data. S.V.B, J.H., D.C.E, H.K. and I.S. wrote the paper and all other  
493 authors discussed and modified the paper.

494



495

496 References

- 497 Anderson PK, Cunningham AA, Patel NG, Morales FJ, Epstein PR, Daszak P. (2004).  
498 Emerging infectious diseases of plants: pathogen pollution, climate change and  
499 agrotechnology drivers. *Trends Ecol Evol* 19: 535–544.
- 500 Aqueveque P, Anke T, Sterner O. (2002). The himanimides, new bioactive compounds from  
501 *Serpula himantoides* (Fr.) Karst. *Z Naturforsch C* 57: 257–262.
- 502 Arantes V, Goodell B. (2014). *Deterioration and Protection of Sustainable Materials* 1158:  
503 3–21.
- 504 Balasundaram SV, Engh IB, Skrede I, Kauserud H. (2015). How many DNA markers are  
505 needed to reveal cryptic fungal species? *Fungal Biology* 119: 940–945.
- 506 Benjamini Y, Krieger AM, Yekutieli D. (2006). Adaptive linear step-up procedures that  
507 control the false discovery rate. *Biometrika* 93: 491–507.
- 508 Berepiki A, Lichius A, Read ND. (2011). Actin organization and dynamics in filamentous  
509 fungi. *Nat Rev Microbiol* 9: 876–887.
- 510 Boddy L, Frankland J, van West P (eds). (2007). Mycelial networks: nutrient uptake,  
511 translocation and role in ecosystems. In: *Ecology of Saprotrophic Basidiomycetes*.
- 512 Bushley KE, Turgeon BG. (2010). Phylogenomics reveals subfamilies of fungal  
513 nonribosomal peptide synthetases and their evolutionary relationships. *BMC Evolutionary*  
514 *Biology* 10: 26.
- 515 Carlsen T, Engh IB, Decock C, Rajchenberg M, Kauserud H. (2011). Multiple cryptic species  
516 with divergent substrate affinities in the *Serpula himantoides* species complex. *Fungal*  
517 *Biology* 115: 54–61.
- 518 De Bie T, Cristianini N, Demuth JP, Hahn MW. (2006). CAFE: a computational tool for the  
519 study of gene family evolution. *Bioinformatics* 22: 1269–1271.
- 520 Eastwood DC, Floudas D, Binder M, Majcherczyk A, Schneider P, Aerts A, *et al.* (2011).  
521 The plant cell wall-decomposing machinery underlies the functional diversity of forest fungi.  
522 *Science* 333: 762–765.
- 523 Emanuelsson O, Brunak S, Heijne von G, Nielsen H. (2007). Locating proteins in the cell  
524 using TargetP, SignalP and related tools. *Nat Protoc* 2: 953–971.
- 525 Fletcher W, Yang Z. (2010). The effect of insertions, deletions, and alignment errors on the  
526 branch-site test of positive selection. *Mol Biol Evol* 27: 2257–2267.
- 527 Floudas D, Binder M, Riley R, Barry K, Blanchette RA, Henrissat B, *et al.* (2012). The  
528 Paleozoic origin of enzymatic lignin decomposition reconstructed from 31 fungal genomes.  
529 *Science* 336: 1715–1719.

530 Floudas D, Held BW, Riley R, Nagy LG, Koehler G, Ransdell AS, *et al.* (2015). Evolution of  
531 novel wood decay mechanisms in Agaricales revealed by the genome sequences of *Fistulina*  
532 *hepatica* and *Cylindrobasidium torrendii*. *Fungal Genetics and Biology* 76: 78–92.

533 Gnerre S, MacCallum I, Przybylski D, Riberio F, Burton JN, Walker BJ *et al.* (2011). High-  
534 quality draft assemblies of mammalian genomes of massively parallel sequence data. *PNAS*  
535 108: 1513–1518.

536 Grime JP, Pierce S. (2012). The evolutionary strategies that shape ecosystems. Wiley-  
537 Blackwell.

538 Grunwald NJ, Goss EM, Press CM. (2008). *Phytophthora ramorum*: a pathogen with a  
539 remarkably wide host range causing sudden oak death on oaks and ramorum blight on woody  
540 ornamentals. *Mol Plant Pathol* 9: 729–740.

541 Harmsen L. (1960). Taxonomic and cultural studies on brown spored species of the genus  
542 *Merulius*. *Friesia* 6: 233–277 pp.

543 Holt C, Yandell M. (2011). MAKER2: an annotation pipeline and genome-database  
544 management tool for second-generation genome projects. *BMC Bioinformatics* 12: 491.

545 Hori C, Ishida T, Igarashi K, Samejima M, Suzuki H, Master E, *et al.* (2014). Analysis of the  
546 *Phlebiopsis gigantea* genome, transcriptome and secretome provides insight into its pioneer  
547 colonization strategies of wood. *PLoS Genet* 10: e1004759.

548 Horton P, Park K-J, Obayashi T, Fujita N, Harada H, Adams-Collier CJ, *et al.* (2007). WoLF  
549 PSORT: protein localization predictor. *Nucleic Acids Research* 35: W585–7.

550 Hulo N. (2006). The PROSITE database. *Nucleic Acids Research* 34: D227–D230.

551 Jennings DH, Bravery AF (eds). (1991). *Serpula lacrymans*: Fundamental Biology and  
552 Control Strategies. Wiley-Blackwell.

553 Jones P, Binns D, Chang H-Y, Fraser M, Li W, McAnulla C, *et al.* (2014). InterProScan 5:  
554 genome-scale protein function classification. *Bioinformatics* 30: 1236–1240.

555 Karlsson M, Durling MB, Choi J, Kosawang C, Lackner G, Tzelepis GD, *et al.* (2015).  
556 Insights on the evolution of mycoparasitism from the genome of *Clonostachys rosea*.  
557 *Genome Biol Evol* 7: 465–480.

558 Kauserud H, Hogberg N, Knudsen H, Elborne SA, Schumacher T. (2004). Molecular  
559 phylogenetics suggest a North American link between the anthropogenic dry rot fungus  
560 *Serpula lacrymans* and its wild relative *S. himantoides*. *Mol Ecol* 13: 3137–3146.

561 Kauserud H, Knudsen H, Hogberg N, Skrede I. (2012). Evolutionary origin, worldwide  
562 dispersal, and population genetics of the dry rot fungus *Serpula lacrymans*. *Fungal Biology*  
563 *Reviews* 26: 84–93.

564 Kauserud H, Svegarden IB, Saetre G-P, Knudsen H, Stensrud O, Schmidt O, *et al.* (2007).  
565 Asian origin and rapid global spread of the destructive dry rot fungus *Serpula lacrymans*. *Mol*  
566 *Ecol* 16: 3350–3360.

567 Klein C, Kuchler K, Valachovic M. (2011). ABC proteins in yeast and fungal pathogens.  
568 *Essays Biochem* 50: 101–119.

569 Kohler A, Kuo A, Nagy LG, Morin E, Barry KW, Buscot F, *et al.* (2015). Convergent losses  
570 of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists.  
571 *Nature Publishing Group* 1–7.

572 Kotlaba F. (1992). Nalezy drevomorky domaci - *Serpula lacrymans* v prirode. *Ceska*  
573 *Mycologie*.

574 Krogh A, Larsson B, Heijne von G, Sonnhammer EL. (2001). Predicting transmembrane  
575 protein topology with a hidden Markov model: application to complete genomes. *J Mol Biol*  
576 305: 567–580.

577 Li L, Stoeckert CJJ, Roos DS. (2003). OrthoMCL: identification of ortholog groups for  
578 eukaryotic genomes. *Genome Res* 13: 2178–2189.

579 Lombard V, Golaconda Ramulu H, Drula E, Coutinho PM, Henrissat B. (2014). The  
580 carbohydrate-active enzymes database (CAZy) in 2013. *Nucleic Acids Research* 42: D490–5.

581 Loytynoja A. (2014). Phylogeny-aware alignment with PRANK. *Methods Mol Biol* 1079:  
582 155–170.

583 Nentwig W (ed). (2008). Biological Invasions. Springer-Verlag.

584 Palfreyman JW, Gartland JS, Sturrock CJ, Lester D, White NA, Low GA, *et al.* (2003). The  
585 relationship between 'wild' and 'building' isolates of the dry rot fungus *Serpula lacrymans*.  
586 *FEMS Microbiol Lett* 228: 281–286.

587 Paradis E, Claude J, Strimmer K. (2004). APE: Analyses of Phylogenetics and Evolution in R  
588 language. *Bioinformatics* 20: 289–290.

589 Parra G, Bradnam K, Korf I. (2007). CEGMA: a pipeline to accurately annotate core genes in  
590 eukaryotic genomes. *Bioinformatics* 23: 1061–1067.

591 Petersen TN, Brunak S, Heijne von G, Nielsen H. (2011). SignalP 4.0: discriminating signal  
592 peptides from transmembrane regions. *Nat Methods* 8: 785–786.

593 Presley GN, Schilling JS. (2017). Distinct Growth and Secretome Strategies for Two  
594 Taxonomically Divergent Brown Rot Fungi. *Appl Environ Microbiol* 83. e-pub ahead of print,  
595 doi: 10.1128/AEM.02987-16.

596 R Development Core Team. (2008) R: A language and environment for statistical computing.  
597 R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org>

598 Rawlings ND, Waller M, Barrett AJ, Bateman A. (2014). MEROPS: the database of  
599 proteolytic enzymes, their substrates and inhibitors. *Nucleic Acids Research* 42: D503–9.

600 Riley R, Salamov AA, Brown DW, Nagy LG, Floudas D, Held BW, *et al.* (2014). Extensive  
601 sampling of basidiomycete genomes demonstrates inadequacy of the white-rot/brown-rot  
602 paradigm for wood decay fungi. *Proc Natl Acad Sci U S A* 111: 9923–9928.

603 Robinson R. (1965). Genetics of the Norway Rat. *International Series of Monographs in*  
604 *Pure and Applied Biology Zoology Division* 24.

605 Sjöström E. (1993). Wood chemistry: fundamentals and applications. Academic Press.

606 Skrede I, Engh IB, Binder M, Carlsen T, Kauserud H, Bendiksby M. (2011). Evolutionary  
607 history of Serpulaceae (Basidiomycota): molecular phylogeny, historical biogeography and  
608 evidence for a single transition of nutritional mode. *BMC Evolutionary Biology* 11: 230.

609 Skrede I, Maurice S, Kauserud H. (2013). Molecular characterization of sexual diversity in a  
610 population of *Serpula lacrymans*, a tetrapolar basidiomycete. *G3 (Bethesda)* 3: 145–152.

611 Stukenbrock EH, Bataillon T, Dutheil JY, Hansen TT, Li R, Zala M, *et al.* (2011). The  
612 making of a new pathogen: Insights from comparative population genomics of the  
613 domesticated wheat pathogen *Mycosphaerella graminicola* and its wild sister species.  
614 *Genome Res* 21: 2157–2166.

615 Syed K, Mashele SS. (2014). Comparative Analysis of P450 Signature Motifs EXXR and  
616 CXG in the Large and Diverse Kingdom of Fungi: Identification of Evolutionarily Conserved  
617 Amino Acid Patterns Characteristic of P450 Family McCluskey K (ed). *PLoS ONE* 9:  
618 e95616–14.

619 Syed K, Shale K, Pagadala NS, Tuszynski J. (2014). Systematic identification and  
620 evolutionary analysis of catalytically versatile Cytochrome P450 monooxygenase families  
621 enriched in model basidiomycete fungi Yu J-H (ed). *PLoS ONE* 9: e86683–18.

622 Syed K, Yadav JS. (2012). P450 monooxygenases (P450ome) of the model white rot fungus  
623 *Phanerochaete chrysosporium*. *Crit Rev Microbiol* 38: 339–363.

624 Tajima F. (1989). The effect of change in population size on DNA polymorphism. *Genetics*  
625 123: 597–601.

626 Watkinson SC, Bebbler D, Darrah P, Fricker M, Tlalka M, Boddy L. (2006). 7 The role of  
627 wood decay fungi in the carbon and nitrogen dynamics of the forest floor. In: Gadd GM (ed).  
628 *Fungi in Biochemical Cycles*. pp 1–31.

629 White NA, Dehal PK, Duncan JM, Williams NA, Gartland JS, Palfreyman JW, *et al.* (2001).  
630 Molecular analysis of intraspecific variation between building and ‘wild’ isolates of *Serpula*  
631 *lacrymans* and their relatedness to *S. himantioidea*. *Mycol Res* 105: 447–452.

632 Yang Z. (2007). PAML 4: phylogenetic analysis by maximum likelihood. *Mol Biol Evol* 24:  
633 1586–1591.

634 Yin Y, Mao X, Yang J, Chen X, Mao F, Xu Y. (2012). dbCAN: a web resource for  
635 automated carbohydrate-active enzyme annotation. *Nucleic Acids Research* 40: W445–51.

636 Yoshida M, Igarashi K, Wada M, Kaneko S, Suzuki N, Matsumura H, *et al.* (2005).  
637 Characterization of carbohydrate-binding cytochrome b562 from the white-rot fungus  
638 *Phanerochaete chrysosporium*. *Appl Environ Microbiol* 71: 4548–4555.

639 Zerbino DR, Birney E. (2008). Velvet: algorithms for de novo short read assembly using de  
640 Bruijn graphs. *Genome Res* 18: 821–829.

641 Zhang J, Presley GN, Hammel KE, Ryu J-S, Menke JR, Figueroa M, *et al.* (2016). Localizing  
642 gene regulation reveals a staggered wood decay mechanism for the brown rot fungus *Postia*  
643 placenta. *Proc Natl Acad Sci U S A* 113: 10968–10973.

644

Figure 1. The dry rot fungus *Serpula lacrymans* and its habitat. *Serpula lacrymans* is one of the most devastating decomposer of houses in temperate and boreal regions worldwide. The species is known to form thick cords and a rapid decay of coniferous wood. In nature the species decompose large logs in dry mountain forests.

Figure 2. The comparative genomic differences among the *Serpula lacrymans* var. *lacrymans*, *Serpula lacrymans* var. *shastensis* and *Serpula himantioides*. A) The number of significantly expanded and contracted gene families, based on analyses using a birth-death model of gene family evolution on all gene clusters. The analyses use a rooted ultrametric tree from a 10 loci maximum likelihood analysis, where *S. himantioides* was the out-group. Thus, only changes in var. *shastensis*, var. *lacrymans* and the branch leading to these two, but not the *S. himantioides* branch were evaluated. B) Phylogenetic sketch trees demonstrating the selection analysis. Each tree highlights a branch and the number of genes with significantly increased or decreased  $\omega$  values on that branch compared to the expected based on 5,866 single gene clusters. The null hypothesis is equal rates on all branches.

Figure 3. Decomposition rate of *Serpula lacrymans* var. *lacrymans*, *S. lacrymans* var. *shastensis* and *S. himantioides* on different wood species. Percent mass loss of wood blocks from the three plant species fir (*Abies lasiocarpa*), pine (*Pinus sylvestris*) and spruce (*Picea abies*) inoculated by var. *lacrymans*, var. *shastensis* and *S. himantioides* for 60 days. No successful growth was obtained for var. *shastensis* on pine.

Table 1. Summary statistics of the genome assembly, annotation and CEGMA analyses of the three sequenced genomes of *Serpula lacrymans* var. *lacrymans*, *S. lacrymans* var. *shastensis* and *Serpula himantioides*

Species	Strain	# of Contigs	# of Scaffolds	N50	Genome size (Mpb)	Assembler	CEGMA	# of predicted genes
var. <i>lacrymans</i>	SL200	4534	1529	59716	37	Velvet	97.6 %	11352
var. <i>shastensis</i>	SHA17-1	3839	1170	92207	38	Velvet	97.2 %	10910
<i>S. himantioides</i> *	MUCL38935	5964	4893	20000	46	AllPathsL	89.5 %	12011 <sup>§</sup>

\*Sequenced by JGI, § Number of genes predicted by Maker annotation tool, however the JGI annotation pipeline predicted 13805 gene models.

Table 2. The gene families that are evolving at a significant different rate (p value < 0.05 after FDR) among the different *Serpula* strains and includes a PFAM domain related to intracellular transport.

Cluster.No	Description	PFAM ID	Test	P value
2435	Domain_of_unknown_function_(DUF202), SPX_domain	PF02656, PF03105	Hl, Lh	0.00899, 0.00044
1272	Cofilin/tropomyosin-type_actin-binding_protein, Variant_SH3_domain	PF00241, PF14604	Hl, Lh	0.03537, 0.00589
6654	RasGEF_N-terminal_motif, RasGEF_domain	PF00618, PF00617	Hl, Lh	0.02021, 0.00370
6080	SNARE_domain	PF05739	Hl, Lh	0.00346, 0.00003
6147	RhoGEF_domain	PF00621	Hl, Lh	0.00899, 0.00573
3843	PX_domain	PF00787	Ll, Hh	0.01602, 0.00220
1940	Oxysterol-binding_protein	PF01237	Hs, Lh	0.01365, 0.00607
3226	PH_domain, FHA_domain, Kinesin_motor_domain	PF00169, PF00498, PF00225	Hs	0.00279
6485	WD_domain, _G-beta_repeat	PF00400	Lh	0.00683
2827	Sec1_family	PF00995	Lh	0.02796
1406	FYVE_zinc_finger, TCP-1/cpn60_chaperonin_family	PF01363, PF00118	Lh	0.01075

H indicates higher omega, L indicates lower omega. *l* symbolizes *Serpula lacrymans* var. *lacrymans*, *s* symbolizes *S. l.* var. *shastensis* and *h* indicates *S. himantoides*, thus Hl indicates significant higher omega for var. *lacrymans*



Table 3. Results from combat experiments with *Serpula lacrymans* var. *lacrymans*, *S. lacrymans* var. *shastensis*, *S. himantioides* and three other fungal species. The proportion of plates with mycelia from the species named in the column after the confrontation test with the species in the row, i.e. read horizontally, higher than 0.5 wins

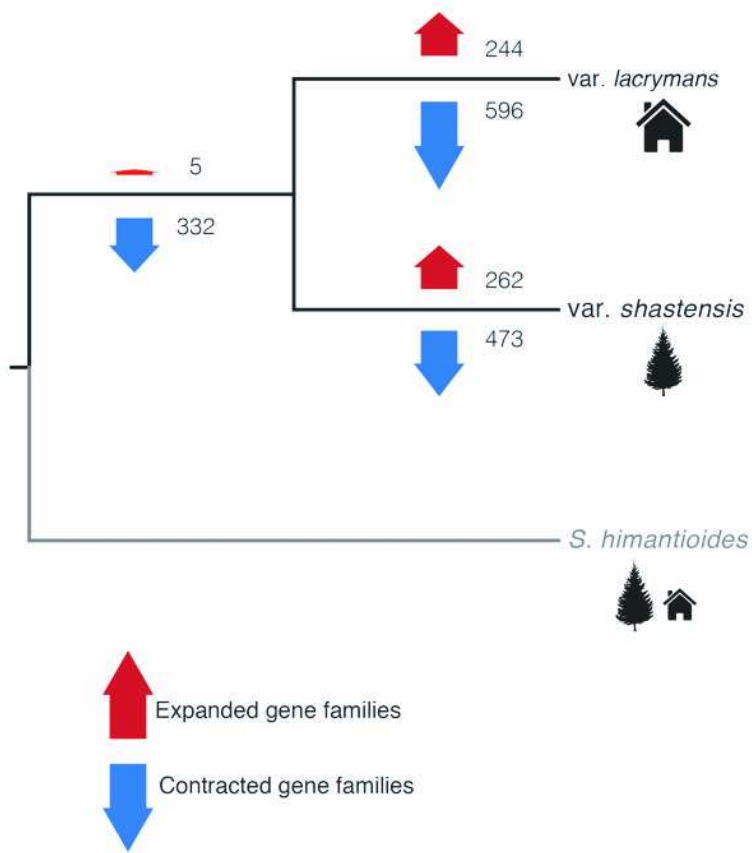
	var. <i>lacrymans</i>	var. <i>shastensis</i>	<i>S. himantioides</i>	<i>C. puteana</i>	<i>A. xantha</i>
var. <i>shastensis</i>	0.450 (20)				
<i>S. himantioides</i>	0.689 (45)*	0.685 (27)			
<i>C. puteana</i>	0.430 (43)	0.500 (34)	0.155 (45)**		
<i>A. xantha</i>	0.400 (45)	0.355 (38)	0.136 (44)**	0.154 (39)**	
<i>F. pinicola</i>	0.978 (46)**	0.889 (45)**	0.156 (48)**	0.931 (29)**	0.292 (48)**

the confrontation with the vertical strain.

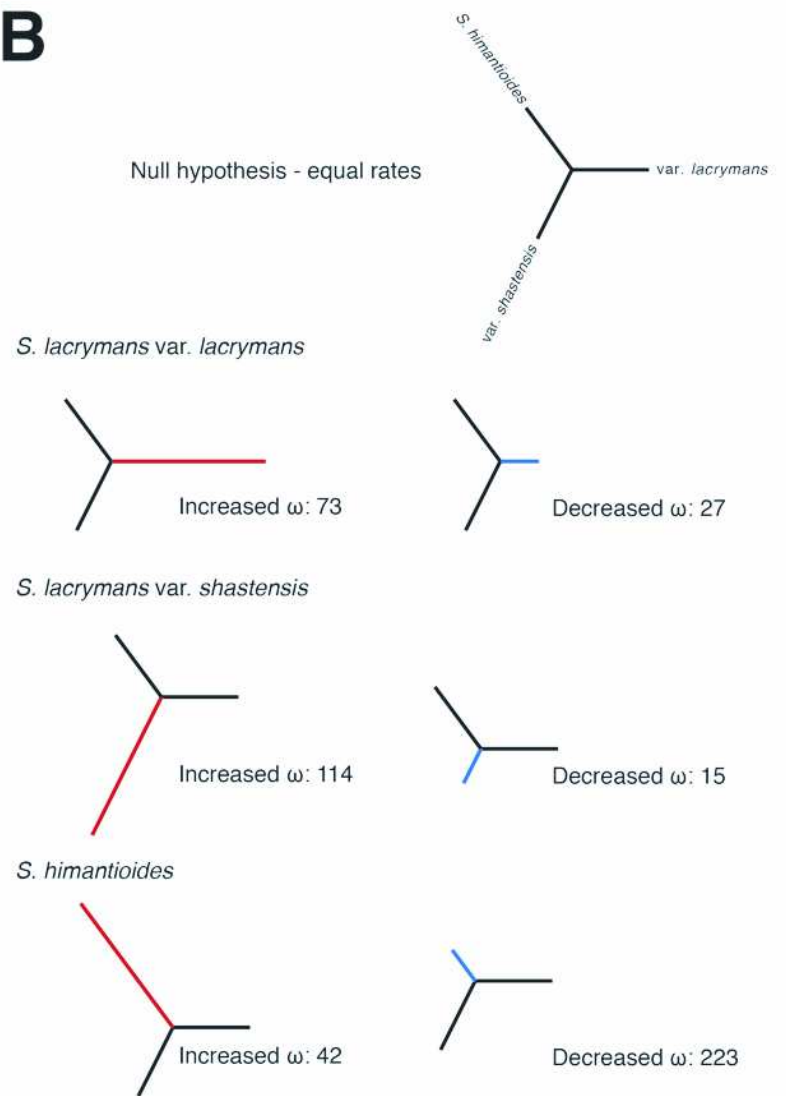
Number of plates (n) used in parenthesis. \* indicates significant different (\*p<0.05, \*\*p<0.005) from expected (E=n/2) by a Person  $\chi^2$  Goodness of fit test, df=1.



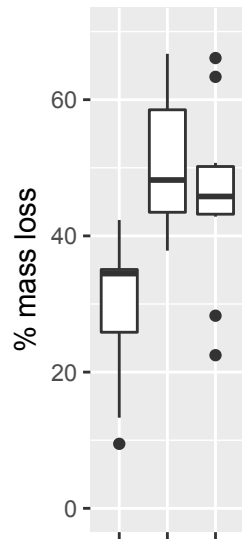
# A



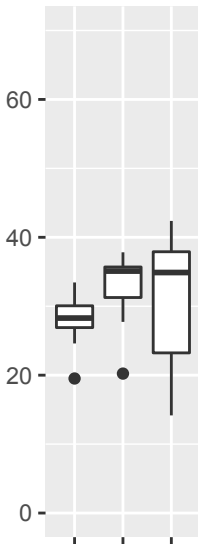
# B



spruce



fir



pine

